Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	18	Ruvkun NEAR Gary	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR .	ON	2005/07/11 08:55
.L2	39	DAF-16	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/07/11 08:55
L3	7323	ELEGANS	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/07/11 08:56
L4	538	AFx fkhr	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2005/07/11 08:56
L5	14	L2 and L3 and L4	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2005/07/11 08:56
L6	65867	insulin	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2005/07/11 08:56
L7	119	L6 and L4	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2005/07/11 08:56
L8	41	L7 and L3	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2005/07/11 08:56
L9	29	(AFx FKHR def-16).clm.	US-PGPUB; USPAT; EPO; DERWENT	OR	ON	2005/07/11 08:56
L10	48	(glucose insulin) AND (afx OR fkhr OR def-16)	US-PGPUB; USPAT; EPO; DERWENT	SAME	ON	2005/07/11 08:56

T.1

L5

(FILE 'HOME' ENTERED AT 08:59:05 ON 11 JUL 2005)

FILE 'MEDLINE, CANCERLIT, AGRICOLA, CAPLUS, SCISEARCH' ENTERED AT 09:02:32 ON 11 JUL 2005
461 S DAF-16
1536 S AFX OR FKHR

L2 1536 S AFX OR FKHR L3 19693 S C. ELEGAN? L4 21 S L1 (L) L2 (L) L3

7 DUP REM L4 (14 DUPLICATES REMOVED)

L6 0 S L5 AND PY<=1997 E RUVKUN GARY?/AU

=> d an ti so au ab 15 1-7

L5 ANSWER 1 OF 7 MEDLINE ON STN DUPLICATE 1

AN 2003538461 MEDLINE

TI Convergence of peroxisome proliferator-activated receptor gamma and Foxol signaling pathways.

SO Journal of biological chemistry, (2003 Nov 14) 278 (46) 45485-91. Electronic Publication: 2003-09-09. Journal code: 2985121R. ISSN: 0021-9258.

AU Dowell Paul; Otto Tamara C; Adi Saleh; Lane M Daniel

AB The forkhead factor Foxol (or FKHR) was identified in a yeast two-hybrid screen as a peroxisome proliferator-activated receptor (PPAR) gamma-interacting protein. Foxol antagonized PPARgamma activity and vice versa indicating that these transcription factors functionally interact in a reciprocal antagonistic manner. One mechanism by which Foxol antagonizes PPARgamma activity is through disruption of DNA binding as Foxol inhibited the DNA binding activity of a PPARgamma/retinoid X receptor alpha heterodimeric complex. The Caenorhabditis elegans nuclear hormone receptor, DAF-12, interacted with the C. elegans forkhead factor, DAF-16, paralleling the interaction between PPARgamma and Foxol. daf-12 and daf-16 have been implicated in C. elegans insulin-like signaling pathways, and PPARgamma and Foxol likewise have been linked to mammalian insulin signaling pathways. These results suggest a convergence of PPARgamma and Foxol signaling that may play a role in insulin action and the insulinomimetic properties of PPARgamma ligands. A more general convergence of nuclear hormone receptor and forkhead factor pathways may be important for multiple biological processes and this convergence may be evolutionarily conserved.

L5 ANSWER 2 OF 7 MEDLINE on STN

DUPLICATE 2

AN 2002664265 MEDLINE

TI Effects of aging and caloric restriction on the gene expression of Foxo1, 3, and 4 (FKHR, FKHRL1, and AFX) in the rat skeletal muscles.

SO Microscopy research and technique, (2002 Nov 15) 59 (4) 331-4. Journal code: 9203012. ISSN: 1059-910X.

AU Furuyama Tatsuo; Yamashita Hitoshi; Kitayama Kazuko; Higami Yoshikazu; Shimokawa Isao; Mori Nozomu

AB In C. elegans, insulin-like hormone signal pathway plays a significant role in longevity. In particular, daf-16 gene product is indispensable factor for this lifespan-extension. This signal pathway is critical for dauer formation, which is a similar state to hibernation in mammals. We examined the expression level of mammalian daf-16 homologues, Foxo 1,3, and 4 (FKHR, FKHRL1, and AFX) mRNAs in the rat skeletal muscles during aging and in 30% caloric restricted of ad libitum fed. The expression level of AFX mRNA was significantly higher at 6 and 12 months than at 3 and 26 months, and FKHRL1 expression was significantly higher at 6 months than at 3 and 26 months but FKHR expression showed no significant change with age. We observed a characteristic expression of AFX and FKHR mRNAs to be significantly higher in the second day in caloric restriction by every-other-day feeding than in ad libitum fed. This suggests that caloric restriction may increase the expression of FKHR-family genes and prevent the aging process in the skeletal muscles. Copyright 2002 Wiley-Liss, Inc.

ANSWER 3 OF 7 L5 MEDLINE on STN

DUPLICATE 3 2001308673 MEDLINE AN

Phosphatidylinositol 3-kinase signaling inhibits DAF-16 DNA binding and TI function via 14-3-3-dependent and 14-3-3-independent pathways.

Journal of biological chemistry, (2001 Apr 20) 276 (16) 13402-10. Electronic Publication: 2000-12-20. Journal code: 2985121R. ISSN: 0021-9258.

ΑU Cahill C M; Tzivion G; Nasrin N; Ogg S; Dore J; Ruvkun G; Alexander-Bridges M

AB In Caenorhabditis elegans, an insulin-like signaling pathway to phosphatidylinositol $\bar{3}$ -kinase (PI 3-kinase) and AKT negatively regulates the activity of DAF-16, a Forkhead transcription factor. We show that in mammalian cells, C. elegans DAF-16 is a direct target of AKT and that AKT phosphorylation generates 14-3-3 binding sites and regulates the nuclear/cytoplasmic distribution of DAF-16 as previously shown for its mammalian homologs FKHR and FKHRL1. vitro, interaction of AKT- phosphorylated DAF-16 with 14-3-3 prevents DAF-16 binding to its target site in the insulin-like growth factor binding protein-1 gene, the insulin response element. In HepG2 cells, insulin signaling to PI 3-kinase/AKT inhibits the ability of a GAL4 DNA binding domain/DAF-16 fusion protein to activate transcription via the insulin-like growth factor binding protein-1-insulin response element, but not the GAL4 DNA binding site, which suggests that insulin inhibits the interaction of DAF-16 with its cognate DNA site. Elimination of the DAF-16/1433 association by mutation of the AKT/14-3-3 sites in DAF-16, prevents 14-3-3 inhibition of DAF-16 DNA binding and insulin inhibition of DAF -16 function. Similarly, inhibition of the DAF-16/14-3-3 association by exposure of cells to the PI 3-kinase inhibitor LY294002, enhances DAF-16 DNA binding and transcription activity. Surprisingly constitutively nuclear DAF -16 mutants that lack AKT/14-3-3 binding sites also show enhanced DNA binding and transcription activity in response to LY294002, pointing to a 14-3-3-independent mode of regulation. Thus, our results demonstrate at least two mechanisms, one 14-3-3-dependent and the other 14-3-3-independent, whereby PI 3-kinase signaling regulates DAF-16 DNA binding and transcription function.

1.5 ANSWER 4 OF 7 MEDLINE on STN **DUPLICATE 4**

AN 2001699838 MEDLINE

Regulation of C. elegans DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway.

so Current biology : CB, (2001 Dec 11) 11 (24) 1950-7. Journal code: 9107782. ISSN: 0960-9822.

AII Lee R Y; Hench J; Ruvkun G AΒ

C. elegans insulin-like signaling regulates metabolism, development, and life span. This signaling pathway negatively regulates the activity of the forkhead transcription factor DAF-16. daf-16 encodes multiple isoforms that are expressed in distinct tissue types and are probable orthologs of human FKHRL1, FKHR, and AFX. We show that human FKHRL1 can partially replace DAF-16, proving the orthology. In mammalian cells, insulin and insulin-like growth factor signaling activate AKT/PKB kinase to negatively regulate the nuclear localization of DAF-16 homologs (reviewed in). We show that the absence of AKT consensus sites on DAF-16 is sufficient to cause dauer arrest in daf-2(+) animals, proving that daf-16 is the major output of insulin signaling in C. elegans. FKHR, FKRHL1, and AFX may similarly be the major outputs of mammalian insulin signaling. daf-2 insulin signaling, via AKT kinases, negatively regulates DAF-16 by controlling its nuclear localization. Surprisingly, we find that daf-7 TGF-beta signaling also regulates DAF-16 nuclear localization specifically at the time when the animal makes the commitment between diapause and reproductive development. daf-16 function is supported by the combined action of two distinct promoter/enhancer elements, whereas the coding sequences of two major

DAF-16 isoforms are interchangeable. Together, these observations suggest that the combined effects of transcriptional and posttranslational regulation of daf-16 transduce insulin-like signals in C. elegans and perhaps more generally.